in weight. Fourteen days following the first stage operation he was given ether and the prostate enucleated. Within twenty minutes the patient had been returned to bed and was in excellent condition. On the fifth day following the operation the temperature and respiration were normal and the pulse was 110 and somewhat irregular. There was no hemorrhage; the urine was not bloody. The large suprapubic drainage tube had been removed and a smaller one had been instituted. The patient was rational. Respirations were thirty. There was a certain amount of dyspnea present. Twelve hours later the dyspnea was very great. The pulse was very rapid and thready. The apex-beat was diffuse and the patient had labored breathing. There was edema of the ankles present. Patient was given stimulation and water was increased as much as he would take. The dyspnea increased rapidly and the heart action became weaker and death occurred the following day. At the autopsy the diagnosis was mitral endocarditis with insufficiency. Chronic myocarditis, edema of both lungs, chronic passive congestion of the liver and spleen and chronic nephritis.

The case records which are quoted here are typical of their class and the autopsy findings are representative of what is found as a cause of death. No effort has been made to determine the mortality rate. The death-rate from prostatectomy has not been higher than with other individuals and institutions. More recently there have been fewer deaths, and I have attributed it to a better understanding of the presence of infection in the form of pyelonephritis which threatens prostatics, and to a prolonged, free, suprapubic drainage, and treatment for infection in their preparation for operation.

URINARY ANTISEPSIS: A STUDY OF THE ANTISEPTIC PROPERTIES AND THE RENAL EXCRETION OF 204 ANILIN DYES.[1]

By Edwin G. Davis, M.D.,

OMAHA, NEB.

In 1915 Hinman[2] pointed out the inefficiency of the several drugs at present in common use as urinary antiseptics and concluded that there is no known drug ideally suited for this purpose or even approaching the ideal. These observations are borne out by the experience of everyone who has had to deal with chronic infections of the urinary tract and also by the absence in the literature of conclusive experimental or clinical evidence of the fitness of any drug

[1] Investigations were carried on in the laboratory of the University of Nebraska College of Medicine, with the aid of an appropriation made by the United States Interdepartmental Social Hygiene Board.

[2] Urinary Antisepsis: A Clinical and Bacteriological Study, Jour. Am. Med. Assn., 1915, lxv, 1769.

for this purpose. The ideal internal urinary antiseptic should be chemically stable and relatively non-toxic and non-irritating; should be antiseptic in high dilution in urine as well as on agar (regardless of the reaction of the former), and should be eliminated in high percentage by the kidney without injury to the body. Clinically there is no such drug known.

For the purpose of urinary antisepsis, urotropin is the most widely used, and likewise the best suited available drug, on account of its well-known and proved action of liberating formalin in the urine. Urotropin, however, has very definite limitations, owing largely to the necessity for an acid urine for the liberation of formalin, and this becomes an insurmountable obstacle in those urines infected with alkalinizing organisms, such as the micrococcus urea or bacilli of the proteus group. As is well known, by the administration of acid sodium phosphate it is possible to cause a temporary slight increase in urinary acidity.

Henderson and Palmer[3] have shown, however, that even after the administration of 10 grams of acid sodium phosphate at a single dose there follows only a slight increase in the hydrogen ion concentration, and in no case were they able to produce a urinary acidity greater than that which they had commonly observed in patients to whom no drug had been administered. On the other hand to produce and maintain a relatively large variation toward the alkaline end of the hydrogen ion scale, by the use of sodium bicarbonate, is comparatively simple. Therefore, granting that the efficiency of a given urinary antiseptic must necessarily be dependent upon the reaction of the urine, a drug efficient in alkaline urine only would be of greater practical value than is urotropin.

Another recognized limitation to urotropin is the time necessary for formalin liberation (Burnam[4]), which destroys the value of the drug at the kidney level and also in the bladder in cases in which for any reason there is rapid emptying or where a fistula exists. The main value of urotropin lies in its use as a prophylactic before instrumentation.

Summary of Previous Studies. Previous publications record the results of investigations with reference to the synthesis of an internal urinary antiseptic, carried on at the Brady Urological Institute, Johns Hopkins Hospital, in coöperation with Dr. Edwin C. White, chemist for that institution. (Davis,[5] Davis and White,[6] Davis,

[3] On the Extremes of Variation of the Concentration of Ionized Hydrogen in Human Urine, Jour. Biol. Chem., 1913, xiv, 81.

[4] An Experimental Investigation of the Value of Hexamethylenamin and Allied Compounds, Arch. Int. Med., 1912, x, 324.

[5] Urinary Antisepsis: A Study of the Antiseptic Properties and Renal Excretion of Compounds Related to Phenolsulphonephthalein: Preliminary Report, Jour. Am. Med. Assn., 1918, lxx, 581.

[6] Urinary Antisepsis: Further Studies of the Antiseptic Properties and Renal Excretion of Compounds Related to Phenolsulphonephthalein, Jour. Urol., 1918, ii, 107.

White and Rosen[7]). Here an attempt was made to correlate chemical structure with the antiseptic properties and the renal excretion of the compounds studied, most of which were synthesized for this special purpose, with the hope that the introduction of certain groups into the molecule would produce certain desired properties. The study was limited largely to compounds related to phenolsulphonephthalein, because of the well-known extraordinary "renal affinity" possessed by this compound and because of its non-toxicity. Some interesting results were obtained which may be briefly summarized as follows:

1. It was possible to establish a certain relationship between chemical structure and renal excretion and to predict the excretion of molecules of certain structure, particularly those of the xanthone group. The halogenation of these compounds interfered with excretion.

2. Many of these compounds, non-toxic, excreted in the urine and antiseptic in water, lost this latter property when tested in voided urine.

3. One compound, chlor-mercury fluorescein, experimentally possessed all of the required properties, and when administered intravenously in minute dosage (5 mgm.) to dogs and rabbits caused the secretion of antiseptic urine for a definite period of time without injury to the animal.

Clinical investigation of this drug has not been carried out on account of its mercury content, although it was shown that in dogs the single lethal dose was forty times that necessary to cause the secretion of antiseptic urine. Chlor-mercury fluorescein, therefore, approaches the ideal in that (*a*) it is antiseptic in high dilution in either acid or alkaline urine; (*b*) it is excreted by the kidney with a rapidity as great as is phenolsulphonephthalein, and (*c*) experimentally efficient dosage may be administered without toxicity. Chlor-mercury fluorescein is an organomercury phthalein derivative in which the mercury is present in non-ionic form.

4. Continued experiments along the same lines (Davis and White[8]) have shown that acriflavin and proflavin are antiseptic in high dilution in urine (particularly in alkalin urine) and that intravenous administration of minute dosage (5 mgm. per kilo) to rabbits causes secretion of urine, which is antiseptic for a definite period of time without injury to the animal. Rabbit urine is normally usually alkaline. Failure to produce antiseptic urine in dogs with corresponding dosage of the same drug was probably due to the fact that dog urine is usually acid (average about p_h, 6).

[7] Urinary Antisepsis: The Secretion of Antiseptic Urine Following the Intravenous Administration of an Organomercury Phthalein Derivative, Jour. Urol., 1918, ii, 277.

[8] Davis, E. G., and White, E. C.: Urinary Antisepsis: The Secretion of Antiseptic Urine Following the Intravenous Administration of Acriflavin and Proflavin, Preliminary Report, Jour. Urol., 1918, ii, 299.

Possibilities Offered by Anilin Dyes. The following record summarizes the results of an investigation of the antiseptic properties and the renal excretion of 204 anilin dyes, the scope of the work being limited and guided, not by the chemical structure of these compounds but only by the number available. This investigation was carried on in the laboratories of the University of Nebraska College of Medicine with the aid of an appropriation made by the United States Interdepartmental Social Hygiene Board.

The anilin dyes were chosen for study (1) because of the large number of these compounds available, (2) because of their color and hence their ready detection and quantitative estimation in the urine and (3) because, through the work of many observers (notably, Churchman,[9] Krumwiede and Pratt,[10] Simon and Wood,[11] Kligler,[12] Graham-Smith[13]), the antiseptic properties of certain anilin dyes have become well known and therapeutic possibilities in this field have been indicated. Furthermore a consideration of the history of the development of the various tests of renal function (Thomas and Birdsal[14]) will call to mind that there are several dyes (fuchsin, rosanilin, indigo-carmin, uranin, trypan blue and others) which have been used to measure the functional activity of the kidneys, and which are therefore known to be excreted without injury to the patient. The staining and penetrating properties possessed by many anilin dyes likewise suggest suitability of this type of compound for medication of the urethral mucosa. This investigation was not undertaken without due realization of the handicap presented by impurities in commercial samples of anilin dyes.

Method of Investigation. Considering the large number of dyes to be studied it was advisable to select a few by preliminary test on agar, thus ruling out many as being unworthy of further investigation. The remaining few were then studied in regard to their antiseptic value in urine, their toxicity, their renal excretion and in regard to their ability to cause the secretion of antiseptic urine following intravenous administration. Finally, those few which were found to be particularly efficient against the staphylococci were tested on special media against the gonococcus. (Tables showing results with the gonococcus will appear in a subsequent publication.) The investigation was therefore divided into five stages as follows:

[9] The Specific Antiseptic Action of Gentian Violet Corresponding to Gram, Jour. Exper. Med., 1912, xvi, 221, 822.

[10] Observations on the Growth of Bacteria on Media Containing Various Anilin Dyes, Jour. Exp. Med., 1914, xix, 20 and 501.

[11] The Inhibitory Action of Certain Anilin Dyes upon Bacterial Development, Am. Jour. Med. Sc., 1914, cxlvii, 247.

[12] A Study of the Antiseptic Properties of Certain Organic Compounds, Jour. Exp. Med., 1918, xxvii, 463.

[13] Some Factors Influencing the Actions of Dyes and Allied Compounds on Bacteria, Jour. Hyg., 1919, xviii, 1.

[14] Comparative Results of Various Functional Renal Tests, Based on a Series of Cases, Jour. Am. Med. Assn., 1917, lxix, 1747.

1. *Antiseptic Values on Agar.* Determination of the antiseptic strength of the entire list of dyes on agar against B. coli, Staphylococcus albus and Staphylococcus aureus.

2. *Antiseptic Values in Urine.* Determination of antiseptic strength of selected dyes in both acid and alkaline urine against B. coli, S. albus and S. aureus.

3. *Toxicity and Excretion.* Determination of toxicity and renal excretion in rabbits of dyes shown to have antiseptic value in voided urine.

4. *Experimental Urinary Antisepsis.* Determination of the antiseptic value of the urine of rabbits which had received intravenous injections of dyes previously shown to be non-toxic and excreted.

5. *Inhibition of Gonococcus.* Determination on special media of the antiseptic strength against the gonococcus of those dyes which had been shown to inhibit the staphylococcus in high dilution.

1. *Antiseptic Values on Agar.* Preliminary antiseptic tests were carried out on the entire list of dyes, using agar neutral to phenolphthalein and of the following composition:

Agar	15 gm.
Peptone (Witte)	10 gm.
Meat extract (Liebig)	5 gm.
Sodium chloride	5 gm.
Water	1000 c.c.

Since the colon bacillus is by far the most frequent invader of the urinary tract, this organism was chosen together with S. albus and S. aureus. The agar was autoclaved in test-tubes in 9 c.c. amounts, after which 1 c.c. of an aqueous solution of the dye was added, the latter solution being at a concentration ten times that desired for the final dilution. Each dilution was then plated, cooled and inoculated by three parallel strokes from twenty-four-hour broth cultures of the three above-named organisms. No concentrations greater than 1 to 1000 were used, all dyes not showing antiseptic properties at this concentration being discarded. The selective antiseptic action against various organisms which Churchman has described, with particular reference to gentian violet, was exhibited by no less than 44 dyes, and in every case it was the colon bacillus that survived, while one or the other of the staphylococci (usually both) failed to grow. (See Figs. 1, 2 and 3.)

2. *Antiseptic Values in Urine.* As previous publications on this subject have indicated the possession of antiseptic properties by a drug when diluted in water or in agar is no indication whatever of its antiseptic value when diluted in urine. Many of the sulphonephthaleins which were germicidal in high dilution in water lost this property when diluted in urine in a test-tube and even permitted the growth of organisms in urine when in relatively high concentration. In determining the antiseptic value in urine of the dyes selected by preliminary test on agar it was therefore desirable to

make the various dilutions with voided urine, since any drug for the above purpose would be useless unless effective in this medium. Furthermore, it was necessary to try out each dye in both acid and alkaline urines, since the ideal drug should be efficient regardless of the urinary reaction.

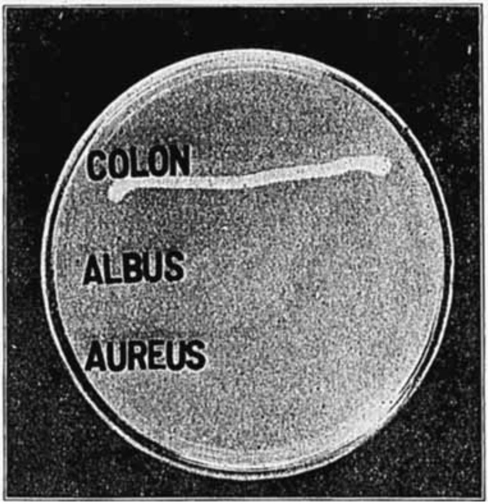

FIG. 1.—Photograph of agar plate containing chrysoidin-Y (1 to 1000), showing selective action of the dye in permitting growth of Bacillus coli and inhibiting Staphylococcus albus and Staphylococcus aureus.

In order to have available each day urine of definite acid and alkaline reaction it was necessary to titrate specimens of voided urine with tenth normal sodium hydroxide and tenth normal hydrochloric acid until definite degrees of hydrogen ion concentration were reached, as determined by the colorimetric method—that is, by comparison with a standard hydrogen ion scale made up with solutions of buffer salts colored by the sulphonephthalein series of indicators. (See publications of Clark and Lubs[15] and Shohl and Janney.[16]) On the acid side of the scale it was arbitrarily decided to use urine titrated to p_h, 6.4, which Henderson and Palmer have shown to be slightly less acid than the average reaction of normal urine. In

15 Colorimetric Determination of Hydrogen Ion Concentration, Jour. Bacteriol., 1917, ii, 1.

16 Growth of Bacillus Coli in Urine at Varying Hydrogen Ion Concentrations, Jour. Urol., 1917, i, 211.

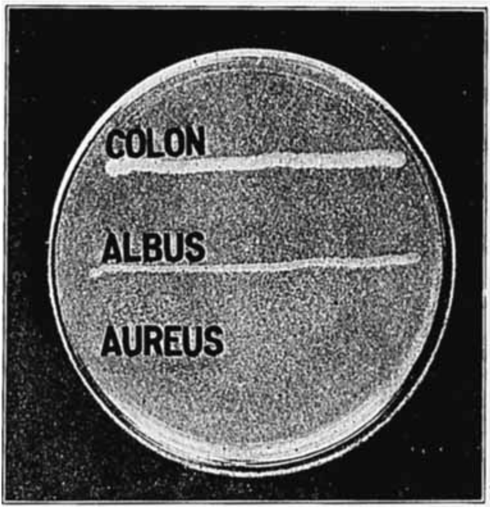

FIG. 2.—Photograph of agar plate containing phloxin-P (1 to 1000), showing selective action of the dye in permitting growth of Bacillus coli and Staphylococcus albus and inhibiting Staphylococcus aureus.

FIG. 3.—Photograph of control plate of drug-free agar, showing profuse growth of all three organisms.

order to obtain alkaline urine a sample of the same specimen was titrated to p_h, 7.6, an end-point arbitrarily chosen so that the reaction of the specimens of urine used from day to day would not vary.

Dilutions of the dyes were made in sterile test-tubes, using acid urine for one series of dilutions and alkaline urine for another. Each dilution was inoculated with one loop of a twenty-four-hour

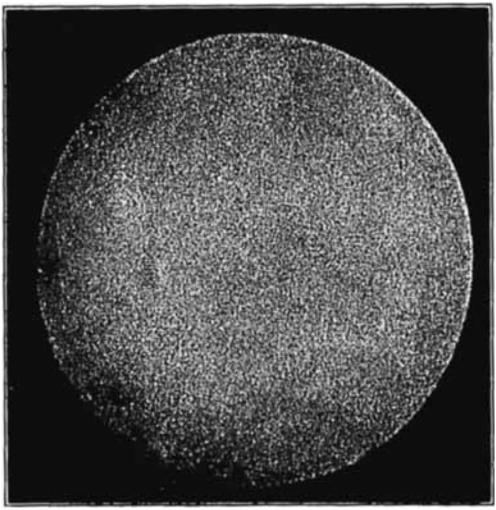

FIG. 4.—Photomicrograph (low power) of typical field in agar plate, showing absence of colonies and proving that the corresponding urine tube had contained a concentration of dye sufficient to kill the organisms during the twenty-four-hour incubation period.

broth culture of Bacillus coli in one series of experiments and with the Staphylococcus albus in another. After an incubation period of twenty-four hours (sufficient time to permit either growth or death of the organism), 0.1 c.c. was transferred from each tube to melted agar and plated. Those plates remaining sterile after incubation (Fig. 4) proved that the urine in the corresponding tubes had contained a concentration of the dye sufficient to kill the organisms within twenty-four hours. Those plates showing a few scattered colonies after incubation (Fig. 5) proved that the concentration of the dye in the urine had been sufficient to cause an arrest in the development of the organisms. This is the "bacteriostatic" action of antiseptics referred to by Hinman and should be

sufficient to control urinary infection provided the administration of the drug is continued. Finally, plates in which countless numbers of colonies developed (designated in the tables by the infinity sign ∞) proved that the concentration of the drug had been insufficient to prevent growth of the organism (Fig. 6).

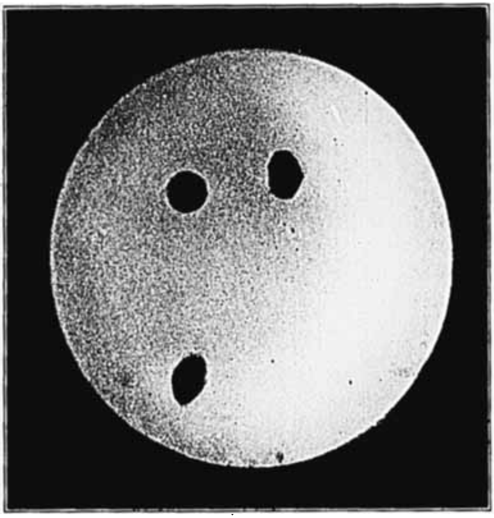

Fig. 5.—Photomicrograph (low power) of typical field in agar plate, showing a few scattered colonies and proving that the corresponding urine tube had contained an inhibitory or bacteriostatic concentration of the dye.

A consideration of Table II permits of several general conclusions. In keeping with the well-recognized clinical frequency of colon bacillus urinary infections, and with the stubbornness of such infections, is the hardiness which this organism displays in urine *in vitro* in spite of the presence of antiseptic dyes. Out of a total of 204 dyes studied only 24 prevented the growth of the colon bacillus in urine in a dilution of 1 to 1000. Of these several were effective in alkaline urine only. A 1 to 1000 solution is a relatively high concentration, considering the extreme dilutions (several greater than 1 to 1,000,000) at which these same dyes are effective against staphylococci in the same media at the same reaction Table II further shows that it is the general rule that these dyes are antiseptic in higher dilution in alkaline than acid urine. This fact might well prove to be of clinical importance, since the artificial production and maintenance of an alkaline urine is a relatively simple matter.

TABLE I.—RESULTS OF PRELIMINARY TESTS ON AGAR OF ANTISEPTIC VALUE OF THE ENTIRE LIST OF DYES AGAINST COLON BACILLUS (C), STAPHYLOCOCCUS ALBUS (Al) AND STAPHYLOCOCCUS AUREUS (Au). ALL DILUTIONS ARE 1 TO 1000. O = GROWTH. — = NO GROWTH.

Name.	No.	Antiseptic strength.			Name.	No.	Antiseptic strength.		
		C.	Al.	Au.			C.	Al.	Au.
NITROSO.									
Naphtholgreen B	4*	O	O	O					
NITRO					NITRO.				
Martius yellow	6	—	—	—	Naphthol yellow S	7	O	O	O
STILBEN.									
Golden yellow	9	O	O	O					
PYRAZOLON.					PYRAZOLON.				
Flavazin L	19	O	O	O	Tartrazin	23	O	O	O
Flavazin S	20	O	O	O					
AZO (MONOAZO).					AZO (MONOAZO).				
Chrysoidin Y	33	O	—	—	Erika B extra	121	O	O	O
Chrysoidin R	34	O	—	—	Erika G extra	122	O	O	O
Soudan I	36	O	O	O	Victoria yel. O	134a	O	O	O
Ponceau 4 GB	37	O	O	O	Metanil yel. extra	134b	O	—	—
Orange G	38	O	O	O	Fast yellow	137	O	O	O
Chromotrope 2R	40	O	O	O	Brilliant yel. S.	142	O	O	O
Orange III	47	O	O	O	Chrysoin	143	O	O	—
Orseillier Mixt.	53	O	O	O	Orange I	144	O	O	O
Chrysoidin	69	O	O	O	Orange II	145	O	O	O
Brilliant orange O	70	O	O	O	Azofuchsin	146	O	O	O
Brilliant orange R	79a	O	O	O	Fast yellow	149	O	O	O
Brilliant orange R	79b	O	O	O	Orange T	151	O	O	O
Palatine scarlet A	81	O	O	O	Naphthyl amino br.	160	O	O	O
Ponceau R	82	O	O	O	Azo rubin	163	O	O	O
Ponceau 3R	83a	O	O	O	Fast red	160	O	O	O
Ponceau 3R	83b	O	O	O	Fast red D	168	O	O	O
Azoeosin G	94a	O	O	O	Cochenille red A	169	O	O	O
Azoeosin	94b	O	O	O	Fast brown 3B	172	O	O	O
Fast red	112	O	O	O					
AZO (DIAZO).					AZO (DIAZO)				
Resorcin brown	211	O	O	O	Vesuvin B	284	O	O	O
Fast brown G	212	O	O	O	Azoalazarin Bord. W.	291	O	—	—
Fast brown	213	O	O	O	Brilliant yellow	303	O	O	O
Blue black	215	O	O	O	Benzopurpurin 4B	363	O	O	O
Palatine black	220	O	O	O	Benzopurpurin 6B	364	O	O	O
Brilliant crocein	227	O	O	O	Rosazurin	372	O	O	O
Soudan IV	232	O	O	O	Azo blue	377	O	O	O
Fast scarlet	248	O	O	O	Dianil blue B	380	O	O	O
Crocein scarlet	249	O	O	O	Trypanblue	391	O	O	O
Crocein scar. 7B	255	O	O	O	Chrysamin R	394	O	O	O
Bismark brown	283	O	—	—	Bavarian blue	450	O	—	—
AURAMINE.					AURAMINE				
Auramine	493	—	—	—	Auramine G	494	O	—	—
TRIPHENYLMETHANE.					TRIPHENYLMETHANE.				
Malachite green	495	—	—	—	Victoria blue 4R	522	O	—	—
Brilliant green	499	—	—	—	Fuchsin S	524	O	O	O
Lt. green SF bluish	504	O	O	O	Red violet 5RS	525	O	O	O
Lt. green SF yellowish	505	O	O	O	Acid violet 4BN	527	O	—	—
Erioglaucine A	506	O	O	O	Acid violet	529	O	O	O
Fuchsin	512	O	—	—	Formyl violet S4B	530	O	O	O
Red violet 5R ex.	514	—	—	—	Methyl blue	538	O	—	—
Methyl violet B	515	—	—	—	Soluble blue	539	O	O	O
Crystal violet	516	—	—	—	Amalga green B	542	O	O	O
Methyl violet	517	—	—	—	Patent blue	543	O	O	O
Ethyl violet	518	—	—	—	Patent blue A	545	O	O	O
Methyl green	519	O	O	O	Cyanol extra	546	O	O	O
Aniline blue	521	O	O	O					

* Numbers correspond to Gustav Schultz, Farbstofftabellen, 1914 (5th edition).

TABLE I.—*Continued.*

Name.	No.	Antiseptic strength.			Name.	No.	Antiseptic strength.		
		C.	Al.	Au.			C.	Al.	Au.
DIPHENYLNAPHTHYL-METHANE.					DIPHENYLNAPHTHYL-METHANE.				
Victoria blue R	558	O	O	O	Wool green S	566	O	O	—
Victoria blue	559	O	—	—					
XANTHONE.					XANTHONE.				
Rodamine S	570	O	—	—	Eosin	587	O	O	O
Rodamine 6G extra	571	O	—	—	Methyleosin	588	O	—	—
Rodamine G extra	572	O	—	—	Eosin BN	590	O	O	O
Rodamine B	573	O	—	—	Erythrosin G	591	O	O	—
Rodamine 3G	576	O	—	—	Erythrosin	592	O	O	—
Sulphorodamine B	579	O	O	O	Phloxine P	593a	O	—	—
Fast acid violet B	580	O	O	O	Phloxine P	593b	O	O	—
Fast acid violet A2R	582	O	O	O	Phloxine	596	O	—	—
Acidrosamine	583	O	O	O	Rose bengale	597a	O	—	—
Fast acid blue	584	O	O	O	Rose bengale extra	597b	O	—	—
Chrysolin	586	O	O	—					
ACRIDINE.					ACRIDINE.				
Benzoflavine	605	O	—	—	Phosphine N	606b	O	—	—
Phosphine	606a	O	—	—	Rheonin	607	O	—	—
QUINOLINE.									
Quinoline yellow	612	O	O	O					
THIOBENZENYL.									
Thioflavine S	615a	O	O	O	Primuline	616	O	O	O
Thioflavine S	615b	O	O	O					
OXAZINE.					OXAZINE.				
Meldola's blue	649	—	—	—	Methylene blue	659	—	—	—
New blue B	650	—	—	—					
New methylene blue	651	O	—	—					
THIAZINE.									
Thiocarmine	662	O	O	O					
AZINE.					AZINE.				
Flavindulin O	668	—	—	—	Safranin MN	683b	O	—	—
Neutral red	670	O	O	O	Nigrosine	698	O	O	O
Indulin scarlet	671	O	—	—	Indulin NN	699	O	O	O
Safranin T	679	O	—	—	Indulin	700	O	O	O
Safranin OW	683a	O	—	—					
MISCELLANEOUS.					MISCELLANEOUS.				
Scarlet 6R	..	O	O	O	Pontacyl pr.	..	O	O	O
Diphenylamino orange	..	O	O	O	Congo red				
Crystal scarlet	..	O	O	O	Scarlet B	..	O	O	O
Biebrich scarlet	..	O	O	O	Pontamine brown R	..	O	O	O
Croceine 3BX	..	O	O	O	Pontamine green GX	..	O	O	O
Guinea green	..	O	O	O	Pontamine green BX	..	O	O	O
Quinone	..	—	—	—	Pontamine black EX	..	O	O	O
Pontacyl sul. blue 5RX	..	O	O	O	Pontachrome yellow	..	O	O	—
Pontacyl ponceau	..	O	O	O	Pontamine orange	..	O	O	O
Pon. sul. acid blue R	..	O	O	O	Methylene blue ZX	..	—	—	—
Pontacyl azo flavine	..	O	O	O	Safranine T extra	..	O	—	—
Pon. sul. black 2B	..	O	O	—	Pontamine yel. SX	..	O	O	O
Pon. blue black SX	..	O	O	O	Primuline	..	O	O	O
Pontamine fast red F	..	O	O	O	Fast yellow NNX	..	O	O	O
Pontamine violet N	..	O	O	O	Erioglaucine	..	O	O	O
Pontamine red B	..	O	O	O	Pontamine fast. yel.	..	O	O	O
Pontamine blue AX	..	O	O	O	Alkali blue	..	O	O	—
Pontamine sky blue 5B	..	O	O	O	Sulphogene ind. blue G	..	O	O	O
Pontamine purpur 10B	..	O	O	O	Aurine	..	O	—	—
Pontamine blue 2B	..	O	O	O	Sulphogene gr. G	..	O	O	—
Ponta. diazo bl. BH	..	O	O	O	Sulphogene or. L	..	O	O	O
Pontachrome black F	..	O	O	O	Thionol black XX	..	O	O	O
Pontachrome bl. 6BX	..	O	—	—	Sulphogene navy blue	..	O	O	O
Hydron blue G	..	O	O	O	Hydron blue R	..	O	O	O
Sulphogene Bordeau B	..	O	O	O	Sulphogene br. G	..	O	O	O
Gentian violet	..	—	—	—					
STANDARD ANTISEPTICS.					STANDARD ANTISEPTICS.				
Mercuric chloride	..	—	—	—	Silver nitrate	..	—	—	—
Phenol	..	O	O	O	Ethyl alcohol	..	O	O	O

TABLE II.—DETERMINATION OF ANTISEPTIC VALUE IN BOTH ACID AND ALKALINE URINE OF 28 DYES SELECTED BY PRELIMINARY TEST ON AGAR FROM THE ORIGINAL 204, AS SHOWN IN TABLE I.

Group.	Dye.	No.	Antiseptic strength in urine.							
			Colon bacillus.				Staphylococcus albus.			
			Acid urine (pH, 6.4).		Alkaline urine (pH, 7.6).		Acid urine (pH, 6.4).		Alkaline urine (pH, 7.6).	
			Inhibits devel. 1 to	Permits growth. 1 to	Inhibits devel. 1 to	Permits growth. 1 to	Inhibits devel. 1 to	Permits growth. 1 to	Inhibits devel. 1 to	Permits growth. 1 to
Nitro	Martius yellow	6	1,000	10,000	1,000	10,000	10,000*	....	10,000*	
Azo	Chrysoidin Y	33	1,000*	10,000	....	1,000	10,000*	....	10,000*	
	Chrysoidin R	34	1,000*	....	....	1,000	30,000	50,000	50,000*	
	Victoria yellow	134b	....	1,000	....	1,000	1,000	10,000	1,000	10,000
Auramine	Auramine	493	....	1,000	1,000*	3,000	1,000	10,000	1,000	10,000
	Auramine G	494	....	1,000	3,000	5,000	1,000	10,000	1,000	10,000
Triphenyl-methane	Mal green	495	5,000*	....	5,000*	....	900,000	1,000,000	1,000,000*	
	Brill. green	499	1,000*	....	1,000*	....	900,000	1,000,000	900,000	1,000,000
	Fuchsin	512	....	1,000	1,000*	....	100,000	300,000	100,000	300,000
	Red viol. 5R ex.	514	1,000*	....	1,000	10,000*	100,000	300,000	700,000*	
	Methyl viol. B	515	3,000	7,000	10,000*	....	1,000,000	....	1,000,000*	
	Crystal viol.	516	1,000	3,000	10,000*	....	1,000,000*	....	1,000,000*	
	Methyl viol.	517	....	1,000	5,000*	....	3,000,000*	5,000,000	9,000,000	10,000,000
	Ethyl viol.	518	....	1,000	3,000	5,000	300,000*	500,000	900,000	1,000,000
	Victoria blue	522	1,000*	....	....	1,000	10,000*	....	90,000	
Xanthone	Rhod. 6G ex.	571	....	1,000	....	1,000	30,000*	....	70,000*	90,000
	Rhod. 3G	576	....	1,000	1,000*	3,000	100,000*	300,000	100,000	300,000
	Rose beng. ex.	597b	....	1,000	....	1,000	30,000	50,000	10,000*	
Acridin	Benzoflavine	605	1,000*	....	1,000*	....	30,000*	70,000	90,000*	
	Rheonin	607	....	1,000	1,000	10,000	10,000*	....	30,000	50,000
Azine	Flavindulin	668	1,000	2,000	1,000	2,000	900,000*	1,000,000	700,000*	900,000
	Indulin scar	671	1,000*	....	1,000*	3,000	1,000	10,000	90,000*	
	Safranin T	679	....	1,000	....	1,000	10,000	30,000	500,000*	700,000
	Safranin OW	683a	....	1,000	....	1,000	10,000	30,000	10,000	70,000
	Safranin MN	683b	....	1,000	1,000*	....	10,000	30,000	100,000	300,000
Miscellaneous	Acriflavine	...	5,000	7,000	100,000	200,000	75,000	100,000	100,000	200,000
	Safranin T ex.	...	....	1,000	....	1,000	30,000*	50,000	90,000*	100,000
	Gentian violet	...	1,000*	....	2,000*	....	100,000	900,000	700,000	900,000
Standard antiseptics	Phenol	...	....	1,000	....	1,000	....	1,000	....	1,000
	Hg. bichloride	...	10,000	30,000	10,000	30,000	10,000	30,000	10,000	30,000
	Ag. nitrate	...	10,000	30,000	10,000*	....	10,000	....	10,000*	

* Indicates that plates showed a few scattered colonies, and that all the organisms were not killed, although there was distinct inhibition of development.

3. *Toxicity and Excretion.* Twenty-seven dyes were selected from Table II as seeming worthy of further study as to toxicity and excretion. Rabbits were given intravenous injections of from 5 to 25 mgm. per kilo and the urine collected at intervals and examined for the dye. Only in those cases in which the excretion was strikingly rapid and complete was an attempt at quantitative colorimetric estimation made. As shown in Table III, several dyes (malachite green, brilliant green, crystal violet, ethyl violet, victoria blue and others) were exceedingly toxic, causing convulsions and death within a few minutes. Autopsies showed a varying and bizarre selective distribution of the different dyes through the various tissues of the body. Several dyes (for instance chrysoidin R, crystal violet, rhodamin 3G, benzoflavin, indulin scarlet and others), though not fatal, were ruled out on account of hematuria or hemoglobinuria following minute dosage. Particular attention is called to the triphenylmethane group, of which many showed antiseptic properties in extreme dilution in urine. Dyes of this group, however, were also the most toxic, and those few which did not injure the rabbit failed to appear in the urine. (See Table III.)

TABLE III.—EXCRETION AND TOXICITY. RESULTS OF INTRAVENOUS INJECTIONS IN RABBITS OF DYES SHOWN IN TABLE II TO POSSESS ANTISEPTIC VALUE *IN URINE.*

Group.	Dye.	No.	Effect on animal. Dose, mgm. per K.	Effect on animal. Result.	Renal excretion. Dose, mgm. per K.	Renal excretion. Result.
Nitro	Martius yellow	6	10	None	10	Moderate.
Azo	Chrysoidin Y	33	20	None	20	Marked.
	Chrysoidin R	34	20	Hematuria	20	Moderate.
	Victoria yellow	134b	20	None	20	Moderate.
Auramine	Auramine	493	10	None	10	Moderate.
	Auramine G	494	10	None	10	None.
Triphenyl-methane	Malachite green	495	20	Lethal	4	None.
	Brilliant green	499	10	Lethal	4	None.
	Fuchsin	512	20	None	20	None.
	Red viol. 5R extra	514	20	Lethal	15	None.
	Methyl violet B	515	30	Anuria	20	None.
	Crystal violet	516	4	Hematuria*	4	None.
	Methyl violet	517	40	Lethal	20	None.
	Ethyl violet	518	20	Lethal	8	None.
	Victoria blue	522	8	Lethal	8	None.
Xanthone	Rhod. 6G extra	571	20	Lethal	10	Moderate.
	Rhod. 3G	576	20	Hematuria	10	Moderate.
	Rose beng. extra	597b	20	None	20	None.
Acridin	Benzoflavine	605	10	Hematuria	4	Slight.
	Rheonin	607	20	None	20	None.
Azine	Flavindulin	668	40	Lethal	20	Doubtful.
	Indulin scar.	671	10	Hematuria	10	Marked.
	Safranin T	679	20	None	20	Moderate.
	Safranin OW	683a	20	None	20	Marked.
	Safranin MN	683b	20	None	20	Marked.
Miscellaneous	Safranin T extra	...	30	None	20	Marked.
	Acriflavine	...	20	None	5	Marked.
	Proflavine	...	20	None	5	Marked.

* Hematuria lasted three days.

4. *Experimental Urinary Antisepsis.* Table III shows that of the total of 204 dyes studied there remained only 13 which were antiseptic in urine (*in vitro*), which were excreted by the kidney after intravenous administration and which exhibited no toxic properties following moderate dosage (about 20 mgm. per kilo). These dyes are listed in Table IV. It then remained to attempt to demonstrate antiseptic properties in the urine of rabbits following the intravenous administration of these drugs; that is, to determine whether passage through the blood stream and kidney would interfere with the antiseptic properties, and whether sufficient dosage could be safely administered to cause adequate concentration in the urine.

TABLE IV.—EXPERIMENTAL URINARY ANTISEPSIS. RESULTS OF ATTEMPTS TO CAUSE THE SECRETIONOF ANTISEPTIC URINE BY THE INTRAVENOUS ADMINISTRATION TO RABBITS OF DYES, SHOWN IN TABLES II AND III TO BE ANTISEPTIC, EXCRETED AND RELATIVELY NON-TOXIC. C = COLON BACILLUS. Al = STAPHYLOCOCCUS ALBUS. ∞ = AN INFINITE NUMBER OF COLONIES. O = NO COLONIES.

Group.	Dye.	No.	Dose. mgm. per K.	Number of colonies which developed in agar plate containing 0.1 c.c. of urine which had previously been inoculated and incubated twenty-four hours.							
				Urine obtained just before injection.		Urine obtained 2 hours after injection.		Urine obtained 6 hours after injection.		Urine obtained 12 hours after injection.	
				C.	Al.	C.	Al.	C.	Al.	C.	Al.
Nitro	Martius yellow	6	10	∞	∞	∞	∞	∞	∞	∞	∞
Azo	Chrysoidin Y	33	20	∞	∞	∞	∞	∞	∞	∞	∞
	Chrysoidin R	34	20	∞	∞	∞	∞	∞	∞	∞	∞
	Victoria yellow	134b	20	∞	∞	∞	∞	∞	∞	∞	∞
Auramine	Auramine	493	10	∞	∞	∞	∞	∞	∞	∞	∞
Xanthone.	Rhod. 6G extra	571	10	∞	∞	∞	∞	∞	∞	∞	∞
	Rhod. 3G	576	10	∞	∞	∞	∞	∞	∞	∞	∞
Azine	Flavindulin	668	20	∞	∞	∞	∞	∞	∞	∞	∞
	Indulin scar	671	4	∞	∞	∞	∞	∞	∞	∞	∞
	Safranin T	679	20	∞	∞	∞	∞	∞	∞	∞	∞
	Safranin OW	683a	20	∞	∞	∞	∞	∞	∞	∞	∞
	Safranin MN	683b	20	∞	∞	∞	∞	∞	∞	∞	∞
Miscellaneous	Safranin T extra	..	30	∞	∞	∞	∞	∞	∞	∞	∞
	Acriflavine	..	10	∞	∞	0	0	0	0	∞	∞
	Proflavine	..	10	∞	∞	0	0	0	0	∞	∞

In general the method of procedure was to compare the antiseptic properties of several specimens of urine, obtained by catheterization from a given rabbit before and at intervals of from one to several hours after drug administration. The necessity for a control urine has been pointed out in a previous publication (Davis and Hain[17]),

[17] Urinary Antisepsis: The Antiseptic Properties of Normal Dog Urine, Jour. Urol., 1918, ii, 309.

which shows that normal dog and rabbit urine, for undetermined reasons, may occasionally act as an unfavorable culture medium for the colon bacillus and may even kill this organism after several hours. Each experiment was therefore accurately controlled by a specimen of urine obtained just before administration of the drug and inoculated and subjected to identically the same conditions as those specimens obtained at intervals after injection.

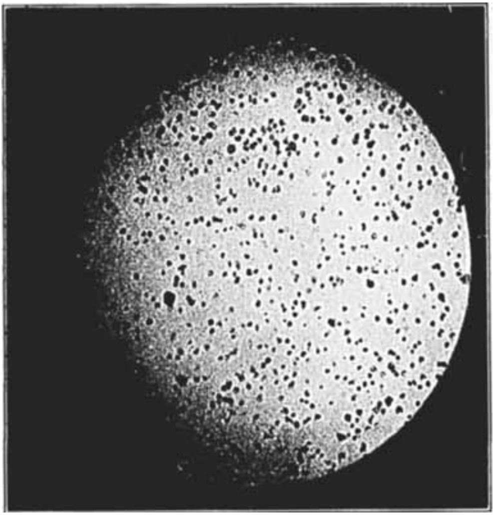

FIG. 6.—Photomicrograph (low power) of typical field in agar plate, showing a countless number of colonies and proving that the corresponding urine tube had contained an insufficient concentration of dye to prevent growth of the organism. The number of colonies in such a plate is designated in Table IV by the infinity sign ∞.

Two samples (1 c.c. each) were transferred from each specimen of urine to sterile test-tubes, inoculated with B. coli and S. albus respectively (one loop of a twenty-four-hour broth culture) and incubated twenty-four hours, after which 0.1 c.c. was transferred from each tube to melted agar and plated. This technic was identical with that described in a preceding paragraph for determining the antiseptic properties of the same dyes in voided human urine, with the exception that the dyes were not added to the urine but were excreted by the kidney. The plates were inspected after forty-eight hours. A plate showing no colonies (Fig. 4) or

very few colonies (Fig. 5) proved that particular specimen of urine to be antiseptic, while the presence of countless numbers of colonies (illustrated by Fig. 6 and designated in Table IV by the infinity sign ∞) proved that the organism had grown and developed and that the urine had acted as a favorable culture medium. This method, dependent upon observing the number of colonies in an agar plate, accurately determines whether the organism has developed or died during the incubation period and is not open to fallacy, as is the method dependent upon gross inspection of incubated urine.

Table IV summarizes the results of attempts to demonstrate antiseptic properties in rabbit urine following the intravenous administration of the 13 dyes shown by previous selection (Tables I, II and III) to be antiseptic, excreted and non-toxic in moderate dosage. The same table shows the results of similar experiments carried out with proflavine and acriflavine. As a previous publication has pointed out (Davis and White[8]), and as the above experiments verify, proflavin and acriflavin are experimentally successful in that antiseptic properties in the urine may be definitely demonstrated following intravenous administration. (The flavins giving these results were manufactured by the Boots Pure Drug Company, Nottingham, England.) Out of the entire list of 204 new dyes, however, although preliminary experiment justified the final selection of thirteen, the properties of which indicated their possible value as internal urinary antiseptics, with none excepting proflavin and acriflavin, was it possible to cause the secretion of antiseptic urine by intravenous administration. In spite of the fact that these several selected dyes approached the ideal requirements (that is were antiseptic in urine, were excreted by the kidney and were relatively non-toxic), yet they failed at the final test when passed through the blood stream and kidney.

Conclusions.—1. There is no known drug ideally suited for the purpose of internal urinary antisepsis.

2. Of a total of 204 anilin dyes investigated 61 were found to possess antiseptic properties in agar, and 28 of these were efficient as antiseptics when added to voided urine.

3. As regards selective action against various organisms, this property was exhibited by no less than 44 dyes, in every case the colon bacillus proving more resistant than the staphylococci. There were only 24 which inhibited the colon bacillus in urine in a dilution of 1 to 1000.

4. There was almost no exception to the rule that antiseptic action was exhibited in higher dilution in alkaline urine than in acid urine. Attention is therefore called to the fact that these dyes are most efficient in urine of a reaction which renders urotropin inert.

5. The azo dyes give no promise of value, since of 37 of this group studied only 3 possessed antiseptic properties, and these only to a slight degree.

6. Of the triphenylmethanes many were antiseptic in high dilution in urine (some in dilution greater than 1 to 1,000,000). Of these, however, all but one were toxic and none was excreted by the kidney. This group is, nevertheless, worthy of further investigation.

7. Of 21 dyes of the xanthane group 3 were antiseptic in voided urine, and 2 of these were excreted to a moderate degree.

8. Of 4 acridine dyes 2 were antiseptic in urine. Neither was excreted.

9. Of 9 dyes of the azine group 5 were antiseptic in urine, and 3 of these (Safranin T, Safranin OW, Safranin MN) were excreted by the kidney with great rapidity and completeness and were non-toxic in 20 mgm. per kilo dosage.

10. By a study of 204 anilin dyes, chosen at random, it has been possible to select 15 which are (*a*) antiseptic in urine, (*b*) excreted by the kidney and which are (*c*) relatively non-toxic. With only two of these, however (proflavin and acriflavin) was it possible to demonstrate the secretion of antiseptic urine following intravenous administration.[8]

11. Considering that rapid renal elimination of anilin dyes is not unusual; that there are not a few dyes, relatively non-toxic, which exert a bacteriostatic action when diluted to infinitesimal amounts in voided urine; and that out of 204 dyes it has been possible to select 15 which approach the ideal and 2 which are experimentally effective; it is within reasonable expectation that a dye clinically suited for use as an internal urinary antiseptic may be discovered or synthesized. Experiments to date indicate that dyes of the triphenylmethane, xanthone, acridin and azin groups (particularly) the latter) give more promise of value.

THE FOURTH VENEREAL DISEASE, ULCERATIVE AND GANGRENOUS BALANOPOSTHITIS: WITH CASE REPORT.

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Judging by the dearth of case reports and the rare mention of the so-called "Fourth Venereal Disease," it would seem that the existence of this condition is not a matter of common knowledge among physicians. Since Corbus and Harris[1] first called it to the attention of the profession in this country in 1909 but two others have made case reports in the American journals.[2,3] Some of the

[1] Jour. Am. Med. Assn., May 8, 1909, lii, 1474.
[2] Bond, S. P.: Urol. and Cut. Rev., 1919, xxiii, 211.
[3] Ross, C. F.: Virginia Med. Monthly, xlv, 36.